Attorney Docket No. **IB-1398**
Customer No. 08076

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Bissell et al.		
Serial No.:	09/652,493	Group No.:	1642
Filed:	August 31, 2000	Examiner:	Yu

Entitled: DESIGN OF NOVEL DRUG SCREENS BASED ON THE NEWLY FOUND ROLE OF DYSTROGLYCAN PROTEOLYSIS IN TUMOR CELL GROWTH

DECLARATION OF JUDITH CAMPISI, Ph.D. UNDER 37 C.F.R. § 1.132

#13

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

CERTIFICATE OF MAILING UNDER 37 C.F.R. § 1.8(a)(1)(i)(A)

I hereby certify that this correspondence (along with any referred to as being attached or enclosed) is, on the date shown below, being deposited with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Dated: ~~May 6, 2003~~ 5/2/03 By: Michelle S Chew
Michelle S. Chew

Dear Sir:

1. I, Judith Campisi, am a Senior Scientist at Lawrence Berkeley National Laboratory, Berkeley, CA and a Professor at the Buck Institute for Aging, Novato, CA. My experience is described in the attached Biographical Sketch and includes research in human and rodent cells in culture, animal models, aging, and tumorgenicity.

2. I have reviewed the above-captioned patent application including the specification and claims.

3. In light of the specification, I understand clearly, and I believe that one of ordinary skill in the art of biology, would understand the terms "potential tumorigenicity" and "medium surrounding cells" as used in the present patent application.

4. Based on the information set forth in the specification, I (and I believe that one of ordinary skill in the art of biology) would be able to design an assay for measuring potential tumorigenicity of mammalian (including human) cells as described in the patent application. This assay could be used either a tissue sample or a sample from the medium surrounding the cells, such as cell culture fluid, blood, lymph or the like. The assay would utilize an antibody to a 120-130 kD fragment of alpha dystroglycan. The assay would detect the level of this fragment. Higher levels of the fragment would correspond to higher potential tumorigenicity. A calibration curve could be established using known cell lines of various degrees of tumorigenicity and non-tumorigenic controls. Establishing a calibration curve and correlating this to tumorigenic potential would be a routine procedure that would be carried out in any given laboratory for a specific set of reagents and reaction conditions, according to basic scientific principles. Although some experimentation would be necessary to achieve the results described referred to in this paragraph, such experimentation would be straightforward, given the basic teachings of this patent application and the findings set forth in the patent application.

5. In my experience, a number of *in vitro* cell culture models are generally recognized in the art as correlating to *in vivo* conditions of tumorigenicity or tumorigenic potential. The three dimensional basement membrane assay, employed in the present application, is especially well regarded as predictive of *in vivo* cell growth behavior of tumor cells. Furthermore, the present specification describes nude mouse experiments that further confirm the correlation between the present *in vitro* assay and *in vivo* results. The present specification provides working examples describing the detection of the 120-130 kD alpha dystroglycan fragment in cell culture medium. It further provides results from a reasonable number of cell lines to show the correlation between shedding of this fragment *in vitro* and potential tumorigenicity, as represented in Fig. 2 of the specification. In this particular case, I believe that it is scientifically credible and plausible to extrapolate the detection of a shed dystroglycan fragment found in cell culture medium to the ability to find that same fragment, using similar techniques, in the blood or other tissue of a living animal, including a human.

Furthermore, the teachings of the present specification support this expectation. I have reviewed the inventors' follow up paper, Muschler et al., "A Role for Dystroglycan in Epithelial Polarization: Loss of Function in Breast Tumor Cells," *Cancer Research* 62:7102-7109 (Dec. 2001), and note that they have obtained *in vivo* data in nude mice that correlate with their *in vitro* work. The nude mouse model is generally accepted as a model predictive of human tumor cell behavior.

6. I have reviewed the inventor's conclusions in the specification, in particular the discussion of the shedding of alpha dystroglycan fragments in hyperplasia and tumor cell growth. Specifically, I have read the following:

> "Because alpha and beta dystroglycan are translated as a single polypeptide, it was surprising that alpha-dystroglycan was not detected on the cell surface of many cells when beta dystroglycan was present. We concluded that, by some mechanism, alpha dystroglycan was being shed from the cell surface." (Page 11, first paragraph).
>
> "We believe alpha dystroglycan shedding occurs principally in cells that are reorganizing and growing. Little of such activity occurs in adult tissues, except in cases like the normal processes of mammary gland development, and perhaps angiogenesis. However, such activity would occur on a large scale during hyperplasia or tumor cell growth and the accompanying angiogenesis. Alpha dystroglycan is shed in two forms, one which binds laminin and a smaller portion with no known binding activity. An assay that detects alpha dystroglycan proteolysis would be an assay for the detection of tissue re-organization and cell growth." (Page 13-14)

7. I believe that the weight of scientific evidence favors the statements quoted in Paragraph 6, rather than raising doubt as to the truth of these statements. The reference to "tissue re-organization and cell growth" also applies also to "potential tumorigenicity."

8. I have reviewed the Office Action dated 11/6/02 in this case and the accompanying references: Dermer, "Another Anniversary for the War on Cancer," *Bio/Technology* 12: 320 (1994); Freshney, "Culture of Animal Cells: A Manual of Basic Technique," (1983) Alan R. Liss, Inc., New York, p. 3-4; Wirth et al., Abstract for "Value of prostate-specific antigen as a tumor marker," *Eur Urol* (1993) 24 Suppl 2:6-12; and Tockman et

al., "Considerations in Bringing a Cancer Biomarker to Clinical Application," *Cancer Research* (Suppl.), 52:2711s-2718s, May 1, 1992). I believe that it is a matter of opinion as to what level of scientific proof is necessary before an assay can have some legitimate practical application. While the data presented in the present patent application may not be sufficient for FDA approval of the claimed assay as a clinical diagnostic modality, nonetheless, the description in the patent application establishes a practical utility for measuring potential tumorigenicity. The assay described may presently be used in research laboratories and could be taken forward into further development without undue experimentation. In addition, given the work described in this application, I believe that there is a utility and reasonable expectation of success for an assay for proteolyzed alpha dystroglycan fragments (as recited in claim 22) and for cell surface markers indicative of proteolytic dystroglycan shedding.

9. I understand that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

Dated: 05/01/03 Signed: Judith Campisi

Judith Campisi, Ph.D.

BIOGRAPHICAL SKETCH

Provid the following information for the key personnel in the order listed for Form Page 2.
Follow the sample format on next page for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Judith Campisi	Senior Scientist/Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
State University New York, Stony Brook	B.A	1974	Chemistry
State University New York, Stony Brook	Ph.D.	1979	Biochemistry

A. Position and Honors

Professional Positions:

1980-1984 Postdoctoral Fellow/Instructor, Dana-Farber Cancer Inst./Harvard Univ Med School
1984-1989 Assistant Professor, Department of Biochemistry, Boston Univ. Medical School
1989-1991 Associate Professor, Department of Biochemistry, Boston Univ. Medical School
1991-present Senior Scientist, Life Sciences Division, Lawrence Berkeley National Laboratory
1992-1999 Group Leader, Aging and Cancer, Lawrence Berkeley National Laboratory
1994-1999 Head, Department of Cell and Molecular Biology, Lawrence Berkeley National Lab.
1999-present Head, Center for Research and Education on Aging, Lawrence Berkeley Nat.'l Lab.
2002-present Professor, Buck Institute for Age Research

Research Awards:

1979 &1982 Postdoctoral Fellowships, American Cancer Soc. and National Institutes of Health
1985 Evangeline Athanas Cancer Research Scholar Award, American Cancer Society
1988 Established Investigator of the American Heart Association
1995 MERIT Award, National Institute on Aging
1997 AlliedSignal Award for Research on Aging
1998 Ellison Medical Foundation, Senior Scholar Award
1999 Glenn Foundation Award, Gerontological Society of America
2002 Irving Wright Award of Distinction, American Federation for Aging Research

Selected Professional Service:

1988-1992 Biological & Clinical Aging Review Committee, National Institute on Aging
1991-2000 Scientific Advisory Committee, Tobacco Related Disease Research Program
1992-present Core Faculty, NIA-Summer Training Course in Experimental Aging Research
1993-present Scientific Advisory Board, Alliance for Aging Research
1994-1998 Board of Scientific Counselors, National Institute on Aging
1997 President's Panel on Cancer
1999-2002 National Advisory Council on Aging, National Institutes of Health

Editorial Boards: Aging Cell, Aging Rev, Exp Cell Res, J Anti-Aging Med, J Cell Biochem, J Cell Physiol, J Geront, Mech Ageing Dev, Molec Biol Rep, Regen Med, Science of Aging-KE.

B. Peer-Reviewed and Selected Other Publications Since 2000

Dimri GP, Acosta M, Itahana K, Campisi J. (2000) Regulation of a senescence checkpoint by the E2F1 transcription factor and p14/ARF tumor suppressor. **Molec Cell Bio** 20: 273-285.

Xu W, Haddad M, Angelis K, Shardy D, Bischof O, Campisi J, Stavenezer E, Medrano EE. (2000) SKI represses Smad2/Smad3 to regulate TGF-β response. **Proc Natl Acad Sci USA** 97: 5924-9.

Lin CQ, Singh J, Murata K, Itahana Y, Parrinello S, Liang SH, Gillett CE, Campisi J, Desprez PY. (2000) A role for Id-1 in the aggressive phenotype and steroid hormone response of human breast cancer cells. **Cancer Res** 60: 1332-1340.

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Huang S, Beresten S, Li B, Oshima J, Ellis N, Campisi J. (2000) Characterization of the human and mouse WRN exonuclease. **Nucl Acids Res** 28: 2396-2405.
Hsu HL, Gilley D, Galande SA, Hande MP, Allen B, Kim SH, Li GC, Campisi J, Kohwi-Shigematsu T, Chen DJ. (2000) Ku acts in a unique way at the mammalian telomere to prevent end joining. **Genes & Dev** 14:2807-2812.
Bischof O, Galande S, Farzaneh F, Kohwi-Shigematsu T, Campisi J. (2001) Selective cleavage of BLM, Bloom syndrome protein, during apoptotic cell death. **J Biol Chem.** 276: 12068-12075.
Campisi J. (2001) From cells to organisms: Can we learn about aging from cells in culture? **Exp Gerontol.** 36: 607-618.
Bischof O, Kim SH, Irving J, Beresten S, Ellis NA, Campisi J. (2001) Regulation and localization of the Bloom syndrome protein in response to DNA damage. **J Cell Bio** 153: 367-380.
Kaminker PG, Kim SH, Taylor RD, Zebarjadian Y, Funk W, Morin G, Yaswen P, Campisi J. (2001) TANK2, a new TRF1-associated PARP, causes rapid cell death upon overexpression. **J Biol Chem** 276: 35891-35899.
Yannone SM, Roy S, Chan D, Murphy M, Huang S, Campisi J, Chen D. (2001) Werner syndrome protein, WRN, is regulated and phosphorylated by DNA-dependent protein kinase. **J Biol Chem** 276: 38242-38248.
Itahana K, Dimri G, Campisi J (2001) Regulation of senescence by p53. **Eur J Bioch** 268: 2784-91.
Krtolica A, Parrinello S, Lockett S, Desprez P, Campisi J (2001) Senescent fibroblasts promote epithelial tumorigenesis: A link between cancer and aging. **Proc Natl Acad Sci USA** 98: 12072-77.
Campisi J. (2001) Cell senescence as a tumor suppressor mechanism. **Trnds Cell Bio** 11: 27-31.
Parrinello S, Lin C Q, Murata K, Itahana Y, Singh J, Krtolica A, Campisi J, Desprez P Y (2001) Id-1, ITF-2 and Id-2 comprise a network of helix-loop-helix proteins that regulate mammary epithelial cell proliferation, differentiation and apoptosis. **J Biol Chem** 276: 39213-39219.
Lim CS, Mian IS, Dernburg A, Campisi J. (2001) *C. elegans clk-2*, a gene that limits life span, encodes a telomere regulator similar to yeast telomere protein Tel2p. **Curr Biol** 11: 1706-10.
Oshima J, Huang S, Pae C, Lee L, Campisi J, Schiestl RH. (2002) Lack of WRN facilitates extensive deletion at non-homologous joining ends. **Cancer Res.** 62: 547-551.
Itahana K, Dimri G , Hara E, Itahana Y, Desprez P, Campisi J. (2002) A role for p53 in maintaining and establishing quiescence in human cells. **J Biol Chem** 277: 18206-14.
Rubio MA. Kim SH, Campisi J. (2002) Reversible manipulation of telomerase expression and telomere length: Implications for the ionizing radiation response and replicative senescence of human cells. **J Biol Chem** 277: 28609-28617.
Kim SH, Kaminker PG, Campisi J. (2002) Telomeres, cancer and aging. **Oncogene** 21: 503-11.
Dimri G, Martinez J, Jacobs J, Keblusek P, Itahana K, van Lohuizen M, Campisi J, Wazer D, Band V. (2002) BMI-1 induces telomerase and immortalizes mammary epithelial cells. **Canc Res** 62: 4736-4745.
Krtolica A, Ortiz de Solorzano C, Lockett S, Campisi J. (2002) Quantification of epithelial proliferation in culture with fibroblasts by fluorescence image analysis. **Cytometry** 49: 73-82.
Itahana K, Zou Y, Itahana Y, Martinez JL, Beausejour C, Jacobs J, van Lohuizen M, Band V, Campisi J, Dimri GP. (2003) Control of the replicative life span of human fibroblasts by p16 and the polycomb protein BMI-1. **Molec Cell Biol.** 23: 389-401.
Campisi J. (2003) Cellular senescence and apoptosis: How cellular responses may influence aging phenotypes. **Exp. Gerontol.** 38: 5-11.
Kim SH, Parrinello S, Kim, J Campisi (2003) *Mus musculus* and *Mus spretus* homologues of the human telomere associated protein TIN2. **Genomics** (n press).
Hasty P, Campisi J, Hoeijmakers J, von Steeg H, Vijg J. (2003) Genomic maintenance systems and aging: Lessons from the mouse. **Science** (in press).
Parrinello S, Krtolica A, Samper E, Goldstein J, Melov S, Campisi J (in revision) Low oxygen abolishes the replicative senescence of murine cells.